Dietary Prebiotics and Arachidonic Acid (ARA) Modulate Intestinal Injury Following Acute Dextran Sodium Sulfate Induced Colitis in Formula-Fed Piglets

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Gastrointestinal (GI) disturbances are among the most common health challenges faced by the suckling neonate, and dietary interventions aimed to modulate inflammation and microbial colonization may provide a prophylactic option to enhance GI development. The objective of this experiment was to investigate the effects of supplemental ARA or prebiotics (alone or combined) on intestinal inflammation and the microbiome in an acute dextran sodium sulfate (DSS) colitis model. Day-old pigs were randomized among 4 diets (0.5% arachidonic acid (ARA; control); 4g/L galactooligosaccharide + 4 g/L polydextrose (PRE); 2.5% ARA, and PRE+ARA; n=24/diet, and were fed 3 times per d for 21d. On d17-21 pigs (12 per diet) were treated with 0.625 g DSS/kg BW to induce colitis. Pigs were euthanized on d22 and samples were collected for digesta pH, histology, and systemic cytokines. Supplements did not affect growth. There was a main effect of PRE on decreasing ileal, cecal, proximal and distal colon digesta pH compared with diets lacking PRE (7.4 vs. 7.3 ± 0.03; 6.2 vs 5.8 ±0.06; 6.3 vs 5.7 ± 0.04; 6.4 vs. 6.0 ± 0.04, respectively; $P<0.05$). PRE and DSS both increased laxation scores with highest diarrhea being found in pigs fed PRE and challenged with DSS ($P<0.05$). In addition, pathology assessments (means ± SEM) showed main effects of diet and DSS on epithelial damage, lamina propria inflammation, crypt abscess, crypt dropout, and overall colitis score ($P < 0.05$). In conclusion supplementation of formula with prebiotics significantly modulates laxation, histopathology, and digesta pH.

Introduction

Gastrointestinal (GI) maladies are one of the top causes of neonatal morbidity and mortality in human infants (1) as well as mammalian livestock species (2). An impaired GI microbiota and inflammation in infancy can alter subsequent immune and physiological response to challenges later in life (3). Neonatal nutrition is a critical component in the establishment of normal GI function and this function is vitally important in reducing neonatal morbidity and mortality (4). Gastrointestinal diseases are a source of substantial human medical cost in the United States, with direct and indirect spending estimated at $142 billion per year (5). It is also a major issue for the swine industry, with diarrheal scours being among one of the top causes of death in nursery piglets (6).

The piglet has gained much popularity as a pediatric research model (7,8). The gastrointestinal tract of the pig has proven to be more similar to that of the human than other domestic and research animal species (9,10). Additionally, the developmental age of the pig is more similar to human infants than rodent models (11). Postnatal intestinal development in piglets is more similar to human infants than rodent models, and immunological responses of the mucosal immune system in piglets is quite similar to the human infant (12).

Suckling piglet research serves to provide a foundational understanding of nutritional regulation of the developing gastrointestinal tract of neonatal piglets as well as human infant. This knowledge in neonatal nutrition can be applied in the swine industry to reduce the effects of intestinal maladies pre- and post-weaning that can negatively impact production systems by increasing the farrow to finish interval through decreased feed efficiency and increasing mortality in the herd. As of 2012, the USDA reported that 3.6% of nursery pigs die and of this group, 9% of the piglet death is caused by scours. Scours can lead to failure to thrive which itself has an even greater detrimental effect on survival by eliminating 22.1 % of nursery piglets (6). This is the

second greatest cause of death in the nursery phase and why nutritional interventions during early phase of development are critical. Additionally, without the ability to feed antibiotics, there is an increasing need to understand and provide nutritional intervention. Neonatal nutrition, in the form of mother's milk or milk replacer, provides the sole source of nutrition to the developing neonate in a critical period of growth and development (3). In addition to providing the sustenance for growth and development, neonatal nutrition also provides the foundation of immunological development and health. Proper formulation of formulas when a mother's milk cannot be consumed is critical to the developing neonate during the time of consumption when consequences of inadequate nutrition are most severe. Bioactive nutrient supplementation in formulas has been steadily improving, with long chained polyunsaturated fatty acids (LCPUFA) arachidonic acid (ARA) and docosahexaneoic acid (DHA) now being added in amounts to formula that are found in mother's milk. More recent research has had the focus on prebiotic supplementation into infant formulas, specifically galactooligosaccharides (GOS) and polydextrose (PDX). There is a great need to improve function and efficacy of formula nutrients and bioactive components in preventing inflammation and promoting gastrointestinal health.

The single layer of intestinal epithelial cells serves as a barrier and gatekeeper protecting the underlying mucosal immune system from overstimulation by the abundant gut lumen antigens. The intestinal enterocytes and mucosal immune cells participate in developing immunologic tolerance, and are responsive to environmental cues to maintain intestinal mucosal homeostasis (13). Barrier function is enhanced by the microbial ecosystem which will produce key signaling molecules known as microbial-associated molecular patterns (MAMP; such as peptidoglycan, LTA, LPS, etc.) that will bind to pattern recognition receptors (PRR; such as TLRs, NODs, PGlyRP, etc.) of mucosal cells and allow for an immune response when stimulated by antigens

(7,8). Innate and adaptive immune response in the developing intestine can be modulated by inflammatory transcription factors, PPARy and NFkB, which alter the balance of anti- and proinflammatory cytokines (8,14,15). Furthermore, elements of both barrier function and innate immune response in mucosal epithelium are responsive to dietary nutrients, and dietary associated changes in the intestinal microbiome (16-21).

Prebiotics can help to mediate controlled inflammation and gut barrier function. Prebiotics are selectively fermented non-digestible ingredients that allow specific changes in both the luminal microbial population and their activity to promote intestinal health by being fermented to short chain fatty acids (SCFA) which can help to enhance barrier function (22). Polydextrose (PDX) supplementation in formula-fed piglets can increase Lactobacilli and decrease luminal pH which results from lactic acid concentrations in the colon (23,24). Galactooligosaccharides (GOS) supplementation has been shown to increase counts of *bifidobacteria* and can clinically help to improve conditions in the case of ulcerative colitis (27, 28). Supplementation of prebiotics to formula fed pigs can also reduce recovery time and improve infection-associated symptoms following *Salmonella typhimurium* infections by reducing diarrhea and increasing intestinal epithelium TER (24).

Milk oligosaccharides (MOs) are known most commonly as being prebiotics which are selectively fermented non-digestible ingredient that allow specific changes in both the luminal microbial population and their activity to promote intestinal health through SCFA production (25). MOs are a complex mixture of indigestible carbohydrates with a high degree of structural diversity and represent one of the largest groups of bioactive components in mother's milk and can influence neonatal mucosal and systemic innate immunity (26). Oligosaccharides are the 3rd most abundant component of milk, with approximately 200 molecular species of oligosaccharides being identified

in human milk and are synthesized from D-glucose, D-galactose, D-N-acetylglucosamine, L-fucose, and D-N-acetylneuraminic acid (sialic acid) monomers (23). Porcine milk oligosaccharides (PMO) are beneficial prebiotics to neonatal piglets. Like humans within a lactation, composition and concentration of PMO will change slightly (26). Although research in the past has pointed towards bovine MOs being the closest in composition to human milk, porcine MOs will have a similar profile of HMOs just at lower concentrations than found in humans (29). Intestinal physiopathology of both the human and porcine species has been shown to be very similar and with MO composition having similar profiles, this suggests similar effects on gastrointestinal development of early nutrition in the neonate, whether it be the piglet or the human infant (30). The microbiome of the gut is thought to be heavily influenced by environment due to the low heritability of the microbiome (31). Prebiotics are antiadhesive antimicrobials that serve as soluble decoy receptors, preventing pathogen attachment to the mucosal surface and lowering the risk for viral, bacterial and protozoan parasite infections. In addition, oligosaccharides may modulate epithelial and immune cell responses, reduce excessive mucosal leukocyte infiltration and activation, lower the risk for necrotizing enterocolitis and provide the infant with sialic acid as a potentially essential nutrient for brain development and cognition (30). Diet influences gut colonization (31), and introducing milk as the first source of oral feeding is important for the establishment of health-promoting bacteria and such as bifidobacteria and lactobacilli (32). When prebiotics are added to formulas, research has shown it can change gut metabolic activity, lower pH of stool, and significantly increase bifidobacteria count (33). Preterm infants often suffer from maladies due to a delayed acquisition of the "normal" digestive microflora stemming from restricted enteral feeding and frequent use of antibiotics (35). Prebiotics have been shown to reduce amounts of pathogens found in the gut (34). In addition, Prebiotics have also been shown

to prevent or lessen the effects of gastrointestinal maladies ranging from mucosal injury to full-thickness necrosis and perforation in preterm infants (33). Long-chain polyunsaturated fatty acids (LCPUFA) can also help to mediate inflammatory balance and gut barrier function. LCPUFA contribute to perinatal growth and development. LCPUFA can foster gut health by having direct effects on inflammatory signaling through modulation of transcription factors, and production of eicosanoids which are critical to intestinal epithelial cell proliferation (15,36-38). Modification of dietary LCPUFAs intake greatly impacts membrane structure through incorporation into cellular membranes phospholipids in many tissues, including brain, retina, and the intestine. (39-41). After ischemic injury, polyunsaturated fatty acid ARA can stimulate rapid recovery of gut barrier function and restore baseline levels of permeability (42-44) and can reduce histological lesions (36). There has also been evidence to show that piglets fed formula containing LCPUFA significantly influenced overall bacterial composition and the size of the Bacteroides community (45).

Previous research has shown that LCPUFA and dietary prebiotics independently can improve neonatal response to inflammation (36,41). The goal of this study is to assess the synergistic effects of dietary prebiotics and LCPUFA on the neonate's ability to resolve intestinal inflammation following dextran sodium sulfate (DSS) induced colitis.

Methods

Pigs and study design. Full-term crossbred piglets were vaginally delivered at the daughter nucleus herd in Rocky Mount, NC (Hanor Farms, Inc.) and allowed to suckle colostrum for 24 h, after which they were transferred to the Developmental Nutrition Lab. Pigs were housed individually in an environmentally controlled room (32°C) programmed to a light/dark cycle of 16/8 h. The pigs were trained to suckle from a nipple plates for the first 24 h using control

diets. The pigs were fed at ~60% ad libitum with fresh diet offered 3 times/d for 21 d to achieve growth rates similar to those of sow-fed pigs. Body weights and milk refusals were measured daily along with qualitative diarrhea scores. The experiment was conducted in 3 replicates. All replicates included 8 pigs/dietary treatments and 4 pigs/diet/±DSS (n = 96 piglets total). All animal procedures were approved by the University Institutional Animal Care and Use Committee.

Diets. Pigs were blocked within treatment by weight and litter of origin and randomly allotted to 1 of 4 dietary treatments. The four dietary treatments differ in prebiotic and fatty acid composition:1) formula containing no prebiotic and baseline LCPUFA (CONT); 2) formula enriched with 4g/L GOS + 4g/L PDX (PRE); 3) formula enriched with 2.5% ARA, or 4) formula enriched with both PRE+ARA. The composition of the basal liquid diet was as follows: milk by-products (Milk Specialties Co., Dundee, IL), 93 g/L; fat supplied according to treatments, 45 g/L; vitamin minerals, 12 g/L; (Milk Specialties Co., Dundee, IL) and water, 850 mL/L. The dry matter content of the basal liquid diet was 15% and the calculated chemical analysis was (DM basis) crude protein, 31.1%; lactose, 36%; ether extract, 25%; and total energy, 4.6 Mcal/kg (696 kcal/L); basal diet composition is reported in **Table 1**. A basal diet was patterned after term human infant formula, adjusted to meet the nutrient requirements of neonatal pigs. Concentrations of prebiotic and LCPUFA were selected based upon previous dose-response studies conducted in our laboratory (23, 41). LCPUFA concentrations in the CONT diet match current industry standards while the enriched diet will contain 5X ARA. At the time of reconstitution in water the basal diet was supplemented with oil blends and/or oligosaccharides. Reconstituted formulas were homogenized and refrigerated before feeding.

Dextran Sodium Sulfate (DSS) Induction of Colitis. To induce colitis, 4 piglets per dietary treatment group were selected at random to receive 0.625 g DSS /kg BW /d for five days beginning on d17 of the trial. Pigs not receiving DSS treatment received an equal volume of sterile saline. The DSS dose was determined by a previous dose response study conducted in our lab and was calculated daily based on daily BW. Pigs remained on dietary treatments during the five day DSS challenge. On d22 following dosage with DSS pigs were euthanized by being anesthetized with isoflurane and then exsanguinate. The intestine was removed from 1 m proximal to the ileocecal junction to the anus. Digesta were collected from the ileum, cecum, proximal and distal colon. The pH was determined on digesta from each intestinal segment, and samples were quantitatively distributed into cryotubes and frozen at -80oC for short-chain fatty acid analysis and microbial enumeration (these two analysis are still on-going).

Sampling.

Growth Performance and clinical assessments. Daily clinical evaluations included measurement of BW, feed intake, observations of stool consistency, presence of blood in stool and overall animal well-being. Feces were visually assessed and assigned a consistency score on a daily basis by the same person throughout the study. A laxation score of 0, 1, 2, 3 was recorded to indicate firm, soft but formed, runny, or severe watery diarrhea, respectively.

Histopathological Measurements. Colon tissue were fixed in 10% buffer formalin for 24 h and stored in 70% ethanol until tissue were ready to be sectioned. Four colon tissue cross sections from each animal were embedded in paraffin blocks and stained with hematoxylin and eosin (H&E). Colonic inflammation was scored by board certified pathologist (North Carolina State University, College of Veterinary Medicine, Raleigh, NC) blinded to treatments. Six different

measures were used to assess severity of colitis adapted from Kim et al. (2010). Additionally, an overall colitis score was given to each sample.

Mucosal and Blood Cytokine Profiles. Concentrations of systemic cytokine, TNFα were analyzed by ELISA (Quantikine ELISA kits according to manufacturer's instructions (Thermo Fisher Scientific/R&D Systems, Inc.)

Statistical Analysis. Data were analyzed by two-way ANOVA as appropriate for a 2X2 factorial design (with repeated measures for BW, FI, and diarrhea scores) using SAS (SAS Institute, Cary, NC). When a significant interaction effect was detected, means were separated using a least-significant-difference test.

Results

Growth performance and laxation scores

There were no differences ($P > 0.05$) in initial and final body weights without DSS and with or without DSS, respectively (**Table 2**). There was a highly significant diet by DSS interaction on diarrheal scores ($P < 0.0001$). The interaction is due to increased stool laxation seen in prebiotic fed piglets regardless of LCPUFA addition (**Table 3**). On d16, d17, and d18 there was no significant effect of dietary treatment on diarrhea score. On d19, d20, d21, and d22 diarrhea scores in CONT + DSS treatments were significantly higher ($P < 0.03$) than the CONT group. Additionally, on d19 treatment group PRE + DSS had a significantly higher ($P=0.03$) diarrheal score. On d20 CONT + DSS continued to have a significantly higher diarrheal score when compared to just CONT ($P=0.003$) On d20 ARA + DSS began to have a significantly higher diarrheal score ($P=0.003$) when compared to ARA treatment with this trend continuing on for d21 ($P=0.002$) and d22 ($P=0.004$). (**Table 3**).

Pathology

The severity of colonic inflammation and the effect of supplementation of prebiotics, LCPUFA or both on DSS-induced colitis was evaluated by H & E staining of colon sections (**Figures 1 and 2**). Assessments included the following measures epithelial damage, LP inflammation, crypt abscess, crypt dropout, goblet depletion, hyperplasia, and an overall colitis score. Colon sections from DSS treated animals showed a distorted crypt architecture and infiltration of inflammatory cells into the mucosa and submucosa ($P < 0.01$). There was an overall effect of DSS on colon architecture compared to no DSS treated pigs ($P < 0.01$). Dietary treatments of prebiotics, arachidonic acid and the combination reduced showed trends in reduction of epithelial damage, crypt abscess, crypt dropout, and overall colitis score ($P < 0.1$). Additionally, there were trends for additive effects on the prebiotic plus arachidonic acid combination on crypt architecture in DSS treated animals.

Intestinal pH

Ileal, cecal, proximal colon and distal colon digesta pH was significantly decreased by prebiotics in the diet ($P < 0.01$; **Table 4**). There was also a significant main effect of DSS on digesta pH from the proximal and distal colon ($P < 0.01$). There was no main effect of dietary ARA on digesta pH or an overall interaction of DSS x PRE x ARA ($P > 0.05$). These data are in agreement with previous work showing addition of prebiotics changes the ileal, cecal and colonic pH by increasing organic acid production in the gut.

Cytokine analysis

An ELISA was ran to determine TNF-α concentrations in the blood serum of 3 piglets per treatment with a total of 12 samples ran. Samples were taken on d1 of the DSS challenge (d17 of the trial) and also from d6 of DSS challenge (d22 of the trial) from each of the dietary treatment

groups. There were no significant changes in TNF-α concentrations from d1 to d6 in these animals (data not shown).

Discussion

Nutritional regulation of neonatal gut health is of primary importance for optimization of growth in neonates. It is well documented that nutrition impacts gut development from digestion and absorption, development of homeostatic microbiota and developing a healthy mucosal immune system (3). Previous work from our lab has shown supplementation of neonatal milk with LCPUFA (ARA or eicosapentanoic acid) will enrich phospholipid membranes of cells throughout the body, as well as, protect against intestinal injury and enhance repair following ischemic injury (36, 41). Additionally, polydextrose and galactooligosaccharide decreased intestinal pH as well as alter microbial populations to enhance lactic acid producing bacteria (23). Much research has been done to demonstrate the independent role of bioactive nutrients in altering intestinal health, but these reductive research methods do not represent the complexity of nutrients involved in modulation of intestinal health by mother's milk. Therefore, this study investigated the independent effects on LCPUFA and prebiotic in a colitis challenge model, as well as, synergistic effects of the two nutrients.

To understand the mechanisms in which gut health is modulated by dietary treatment growth performance, colon histological pathology, intestinal pH, and systemic TNF-α were examined. Regardless of treatment, weight gain was not compromised when final body weight was analyzed with or without colitis. Diarrheal scores were altered by dietary treatment with prebiotics which is supported by previous research (23). However, the increased laxation prior to the intestinal challenge may have masked the impact of the DSS challenge on diarrhea in the prebiotic piglets. Increase in laxation scores was seen with all prebiotic treatment groups with or without the addition of ARA before colitis was induced and with worsening effects as the trial went on.

This data suggests that feeding prebiotics at these high amounts could have potential effects of increasing laxation scores of neonatal animals. By day four of the colitis challenge there was increased diarrhea in all dietary treatment groups treated with DSS.

The diarrhea data is supported in the colonic histopathology work where there was an overall increase in colonic damage with DSS treatment, but there were trends for reduced injury of colonic architecture with PRE, ARA or the combination. Epithelial damage and colonic abscesses and crypt dropout showed a trend for improvement following dietary treatment with these bioactive nutrients. Others have shown that prebiotics impact intestinal health (14,23,25) but showing improved gut health with a 5-fold increase in ARA was a first time this has been shown. Additional work is needed to understand the mechanisms of by which these bioactive nutrients may protect against intestinal injury, but it is safe to say there are most likely multiple layers to the effects seen. The lowering the in intestinal pH is most likely correlated with changes in microbial populations in the gut. Herfel et al. (23) showed that increasing concentration of PDX in neonatal pig diets decreased pH while increasing lactic acid and lactic acid producing bacteria. They also showed there were changes in bifidobacterium in the piglet gut, which have been associated with positive gut health in human infants (26). These early population changes are important to establishing a 'homeostatic' microbiome and protecting against the development of a dysbiotic microbiome that could lead to lifelong immune challenges that are correlated with autoimmune diseases, such as diabetes, asthma, irritable bowel syndromes, and others (26). However, further work is needed to optimized dose of prebiotics associated with gut health since animals on the PRE treatments experienced diarrhea prior to intestinal challenge.

Systemic TNF-α analysis proved to be nonsignificant with the small number of samples we have run, but local cytokine analysis and further systemic cytokine analysis must be done

before conclusions can be drawn. Overall, DSS had an effect of increasing damage of colon architecture and dietary treatments with a combination of prebiotic and ARA have the potential to protect the architecture in the colon. In addition, prebiotics proved to drop the intestinal pH to provide an environment for more beneficial bacteria but at the levels fed have a potential negative effect of increasing diarrhea. In conclusion, dietary prebiotics were seen to modulate colon pathohistology and the intestinal microbiome through lowering pH and increasing laxation scores during intestinal injury.

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Table 1 Composition of basal (control) formula fed to newborn pigs for 21 d

Basal Diet[1]	
Ingredient	%
Na caseinate	11.25
Delactosed whey	18.11
Dicalcium phosphate	1.88
Calcium chloride	0.33
Mineral premix[2]	0.50
Vitamin premix[3]	0.12
Artificial flavor	0.03
Potassium sorbate	0.45
D,L Methionine	0.49
Whey	28.59
Whey protein concentrate	18.15
Edible lard	19.39
Sodium hexametaphosphate	0.18
Antioxidant	0.01
Flow agent	0.14
Emulsifier	0.22
Lecithin	0.19
TOTAL	100.00

[1]Manufactured by Milk Specialties, Dundee, IL.

[2]Mineral premix contained: 1 g/100 g Ca, 0.55 g/100 g P, 0.28 g/100 g Na, 0.04 g/100 g Cl, 2.02 g/100 g K, 0.1 g/100 g Mg, 20,000 mg/g Fe, 200 mg/100 g Co, 1850 mg/g Cu, 400 mg/g I, 5000 mg/g Mn, 60 mg/g Se, 23,500 mg/g Zn.

[3]Vitamin premix contained: 9.9 g/kg retinol, 0.17 g/kg cholecalciferol, 55 g/kg α- tocopherol, 117,000 mg/g ascorbic acid, 29,983 mg/g D-pantothenic acid, 33,069 mg/g niacin, 8378 mg/g riboflavin, 5115 mg/g menadione, 66 mg/g biotin, 44000 mg/g vitamin B-12, 2038 mg/g thiamin, 3996 mg/g vitamin B-6, 2756 mg/g folic acid.

Table 2. Growth performance of neonatal pigs fed formulas containing LCPUFA and/or prebiotics for 21 d[1]

	No DSS				Plus DSS					
	CONT	**ARA**	**PRE**	**PRE + ARA**	**CONT**	**ARA**	**PRE**	**PRE + ARA**	**SE**	**P-value**
IBW (g)	1643.43	1655.09	1680.33	1616.79	1526.92	1503.32	1627.83	1546.56	58.0	0.28
FBW (g)	5438.30	5437.83	5447.00	5271.05	5270.75	5160.40	5448.50	5448.50	101.6	0.35

[1]Values ae means and pooled SEMs, n=12. IBW, initial body weight; FBW, final body weight; CONT, control diet; ARA, 2.5% arachidonic acid in the diet; PRE, 4g/L polydextrose + 4g/L galactooligosaccharide; PRE + ARA, 2.5% arachidonic acid + 4g/L polydextrose + 4g/L galactooligosaccharide.

Table 3. Diarrhea scores in piglets fed prebiotics, arachidonic acid, or both with or without a DSS colitis challenge

	No DSS				Plus DSS				P-value				
	CONT	**ARA**	**PRE**	**PRE + ARA**	**CONT**	**ARA**	**PRE**	**PRE + ARA**	**SE**	**DSS*PRE*ARA**	**DSS**	**PRE**	**ARA**
d16	0.12^{c}	0.17^{c}	1.61^{b}	1.93^{a}	0.17^{c}	0.08^{c}	1.44^{b}	2.06^{a}	0.1	< 0.0001	0.88	< 0.0001	0.25
d17	0.2^{d}	0.08^{d}	1.62^{b}	1.94^{a}	0.42^{c}	0.08^{d}	1.67^{b}	1.84^{a}	0.1	< 0.0001	0.65	< 0.0001	0.95
d18	0.37^{c}	0.33^{c}	1.52^{b}	1.98^{a}	0.42^{c}	0.42^{c}	1.69^{b}	1.92^{a}	0.1	< 0.0001	0.59	< 0.0001	0.41
d19	0.11^{e}	0.08^{e}	1.39^{b}	1.92^{a}	0.50^{c}	0.25^{d}	1.76^{a}	1.90^{a}	0.1	< 0.0001	0.15	< 0.0001	0.68
d20	0.37^{d}	0.41^{d}	1.50^{b}	1.99^{a}	1.08^{c}	1.08^{c}	1.77^{a}	2.00^{a}	0.2	< 0.0001	0.009	< 0.0001	0.31
d21	0.46^{c}	0.83^{c}	1.92^{a}	1.89^{a}	1.33^{b}	1.58^{b}	1.99^{a}	1.91^{a}	0.2	< 0.0001	0.004	< 0.0001	0.52
d22	0.46^{c}	0.92^{b}	1.93^{a}	1.82^{a}	1.25^{b}	1.75^{a}	1.99^{a}	1.98^{a}	0.2	< 0.0001	0.002	< 0.0001	0.23

[1]Values ae means and pooled SEMs, n=12. CONT, control diet; ARA, 2.5% arachidonic acid in the diet; PRE, 4g/L polydextrose + 4g/L galactooligosaccharide; PRE + ARA, 2.5% arachidonic acid + 4g/L polydextrose + 4g/L galactooligosaccharide.

Figure 1. Quantitative histological grading of neonatal pig colitis main effect of DSS.

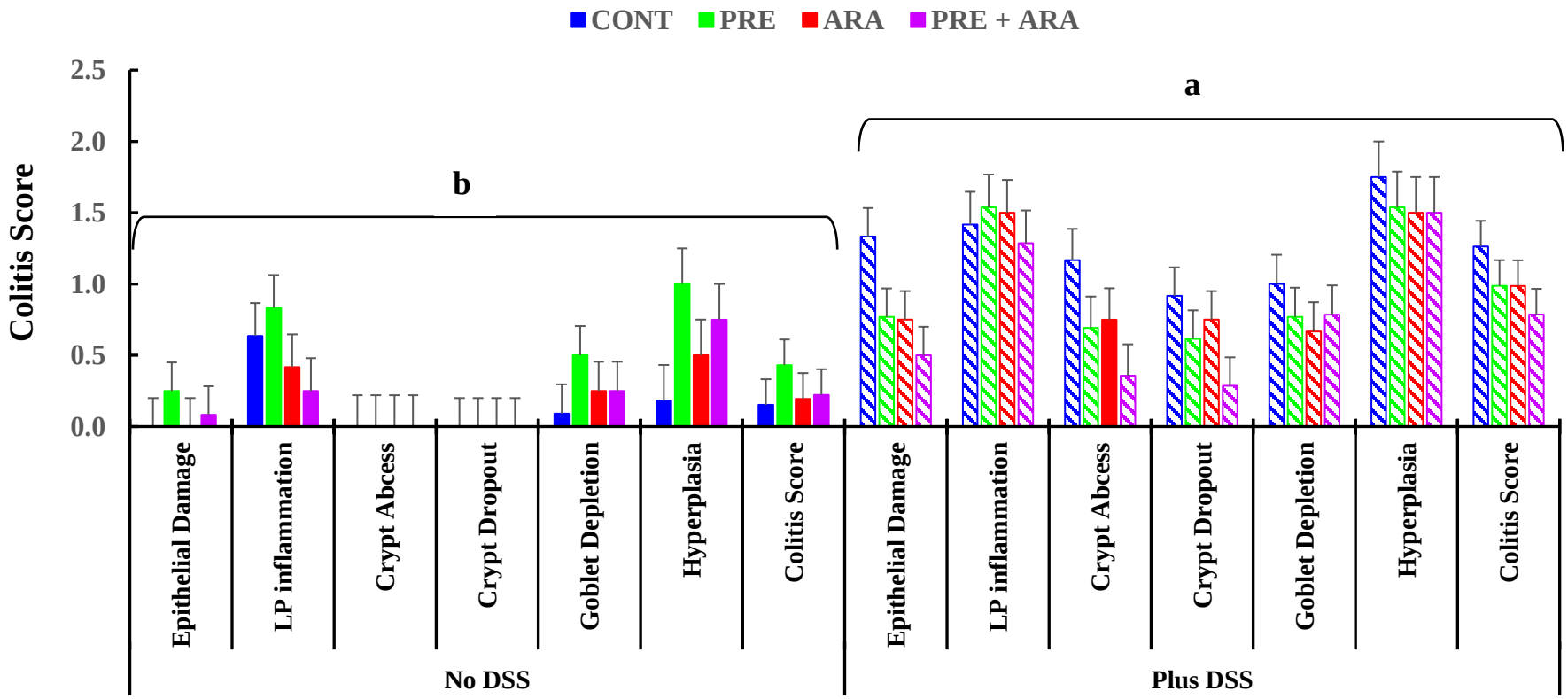

Figure 2. Quantitative histological grading of neonatal pig colitis dietary effects.

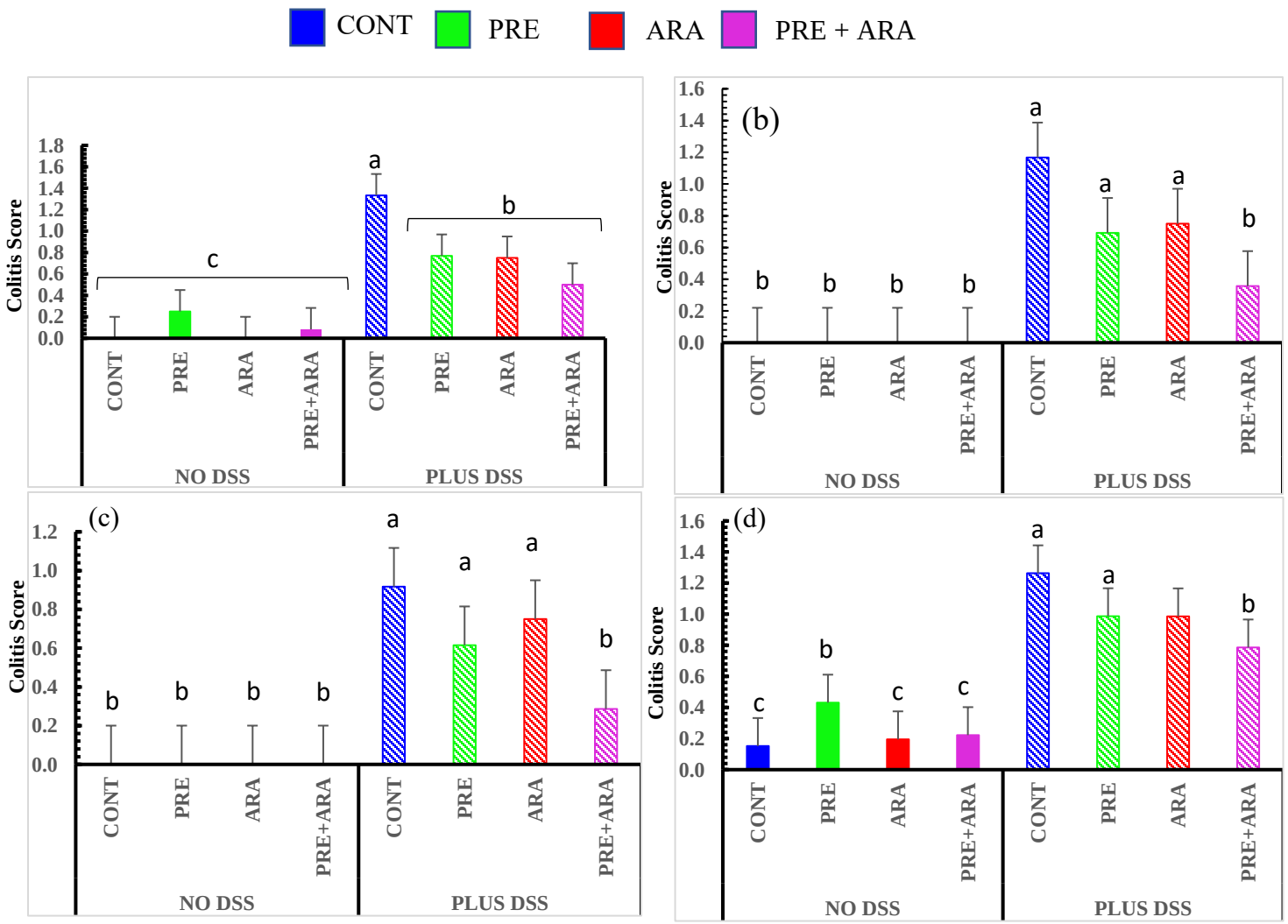

Table 4. Digesta pH in piglets fed prebiotics, arachidonic acid, or both with or without a DSS colitis challenge

	No DSS				Plus DSS				p-value				
	CONT	**ARA**	**PRE**	**PRE + ARA**	**CONT**	**ARA**	**PRE**	**PRE + ARA**	**SE**	**DSS*PRE*ARA**	**DSS**	**PRE**	**ARA**
Ileum	7.36ab	7.36ab	7.29b	7.31b	7.48a	7.4ab	7.32b	7.32b	0.04	0.66	0.12	0.01	0.62
Cecum	6.22[a]	6.24[a]	5.74[b]	5.81[b]	6.27[a]	6.12[b]	5.99[b]	5.83[b]	0.08	0.96	0.40	< 0.0001	0.35
Proximal Colon	6.38[a]	6.34[a]	5.72[c]	5.78[c]	6.21[ab]	6.09[b]	5.75[c]	5.71[c]	0.06	0.47	0.01	< 0.0001	0.44
Distal Colon	6.50[a]	6.61[a]	6.10[b]	6.15[b]	6.22[b]	6.21[b]	5.88[c]	5.92[bc]	0.06	0.90	< 0.0001	< 0.0001	0.22

[1]Values ae means and pooled SEMs, n=12. CONT, control diet; ARA, 2.5% arachidonic acid in the diet; PRE, 4g/L polydextrose + 4g/L galactooligosaccharide; PRE + ARA, 2.5% arachidonic acid + 4g/L polydextrose + 4g/L galactooligosaccharide.